

**Randomized controlled trial on the use of EPO
to reduce top-up transfusions in neonates
with red blood cell alloimmunization treated with intrauterine
transfusions**

Short Title: [EPO-4-Rhesus Study](#)

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1. LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ALAT	Alanine aminotransferase
ASAT	Aspartate aminotransferase
BSID-III	Bayley scales of infant development, third edition
DSMB	Data safety monitoring board
EPO	Erythropoietin
EudraCT	European drug regulatory affairs clinical trials
GCP	Good clinical practice
γGT	Gamma-glutamyl transferase
HDFN	Hemolytic disease of the fetus and newborn
IUT	Intrauterine red cell transfusion
LDH	Lactate dehydrogenase
LUMC	Leiden University Medical Center
MDI	Mental developmental indexes
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
PDI	Psychomotor development indexes
(S)AE	(Serious) adverse event
SUSAR	Suspected unexpected serious adverse reaction
Wbp	Personal data protection act; in Dutch: Wet bescherming persoonsgegevens
WMO	Medical research involving human subjects act; in Dutch: wet medisch-wetenschappelijk onderzoek met mensen

2. SUMMARY

Rationale: neonates with hemolytic disease of the fetus and newborn (HDFN) due to red cell alloimmunization often require top-up (red blood cell) transfusions to treat late anemia during the first 3 months of life. Late anemia in neonates with HDFN may be due to depressed erythropoiesis (hyporegenerative anemia) and/or persisting (intra-marrow) destruction of erythrocytes by remaining antibodies, which is not necessarily associated with parameters of hemolysis. Hyporegenerative anemia occurs in particular in neonates treated with intrauterine red cell transfusions (IUT) due to bone marrow suppression. Approximately 80% of infants with rhesus hemolytic disease treated with IUT require at least one top-up transfusion, with an average of two top-up transfusions per infant.

Since more than a decade erythropoietin (EPO) has been applied in small studies and casuistic reports in children with HDFN. It is controversial whether neonates with HDFN treated with or without IUT benefit from this treatment to reduce the risk of delayed anemia and subsequently, the need for top-up transfusions. Due to the lack of evidence, routine use of EPO is currently not recommended. To determine a role for EPO in patients treated with IUT, a well-designed randomized controlled clinical trial of sufficient sample size is required.

Objective: to evaluate the effect of darbepoetin alfa on the need for top-up transfusions in neonates with HDFN due to red cell alloimmunization treated with IUT. Secondary outcomes are number of days of admission for top-up transfusions, percentage of infants with high ferritin levels and high blood pressure.

Study design: randomized controlled trial.

Study population: all (near)-term neonates (gestational age ≥ 35 weeks) admitted to the Leiden University Medical Center (LUMC) with HDFN, treated with IUT.

Intervention: treatment with darbepoetin alfa subcutaneously at a dosage of 10 $\mu\text{g}/\text{kg}$ once a week for a period of 8 weeks (intervention), or “standard care”.

Main study endpoints: number of top-up transfusions required per infant.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: all infants participating in either treatment arm of the study are subjected

to routine neonatal care. Darbepoetin alfa is given subcutaneously, once a week for a period of 8 weeks on home visits. Administration of darbepoetin alfa may cause local skin reactions, increased blood pressure with rarely convulsions and hypersensitivity reactions. However, these risks are small and are observed in different populations. If effective, darbepoetin alfa prevents or reduces the amount of top-up transfusions. Additionally, darbepoetin alfa may improve neurodevelopmental outcome. No extra blood will be drawn from the infants in addition to the weekly routine measurements of complete blood counts. Blood pressure will be measured three times throughout treatment.

HDFN is a condition occurring exclusively in infants and can therefore only be studied in this specific population.

3. INTRODUCTION AND RATIONALE

The mainstay of antenatal treatment of fetal anemia due to red cell alloimmunization is (serial) intrauterine red cell transfusions (IUT).^{1,2} The mainstay of postnatal treatment in hemolytic disease of the fetus and newborn (HDFN) is (1) intensive phototherapy and exchange transfusion to treat hyperbilirubinemia and prevent kernicterus, and (2) top-up transfusions to treat anemia.³ Up to 80% of infants with HDFN treated with IUT require at least one top-up transfusion for late anemia during the first 3 months of life, with an average of two top-up transfusions and therefore two hospital admissions per infant.³⁻⁵

Late anemia is attributed to two mechanisms: “hyporegenerative anemia” characterized by depressed erythropoiesis⁶ and “late anemia of hemolytic disease” caused by persisting antibodies, destroying newly produced erythrocytes.^{4,7} “Hyporegenerative anemia” occurs in particular in neonates treated with several IUTs due to bone marrow suppression.^{4,6} To distinct these two mechanisms, a persistence of a high antibody titer can be used.⁸

Several risk factors for late anemia have been reported, including serial IUT (due to bone marrow suppression), severity of HDFN, reduced use of exchange transfusions during the neonatal period and reduced survival of transfused red blood cells.³ Finally, erythropoietin deficiency is also considered as a possible contributing factor to late anemia.⁹⁻¹⁴

Erythropoietin (EPO) has been increasingly used in neonates to prevent or reduce neonatal anemia without short or long-term adverse effects.^{13,15-17} Several small studies and casuistic reports suggest that neonates with HDFN may benefit from treatment with EPO to reduce the risk of delayed anemia and subsequent top-up transfusions.⁹⁻¹⁴ However, other authors found that EPO may be less effective than expected.^{8,18} Due to the lack of evidence, routine use of EPO is currently not recommended.³ To determine a role for EPO in this group of patients, a well-designed randomized controlled clinical trial of sufficient sample size is required.¹⁴

Potentially, EPO stabilizes the hemoglobin levels of these infants and thus prevents anemia, hospital admissions for top-up transfusions and potential transfusion reactions, creating a more stable and natural postnatal course for patients with HDFN. In this scenario, the current

management of weekly out-patient visits and weekly blood draws for hemoglobin level measurements, may be reduced, further contributing to reduction of the burden for these infants.

HDFN is a condition occurring exclusively in infants and can therefore only be studied in this specific population.

4. OBJECTIVES

The primary objective of this study is to investigate whether darbepoetin alfa is effective in reducing the incidence of late anemia in infants with HDFN treated with IUT and therefore in decreasing the number of top-up transfusions per infant. As secondary objectives the percentage of infants that required a top-up transfusions will be assessed, as well as the time from birth to first top-up transfusion, number of days of admission for top-up transfusions, percentage of infants with a systolic blood pressure ≥ 2 SD above age adjusted mean systolic blood pressure and the percentage of infants with ferritin levels >200 $\mu\text{g/L}$. For exploratory purposes, the long-term neurodevelopmental outcome at 2 years of age will be assessed using the BSID-III test.

5. MATERIAL AND METHODS

5.1 Study population

All (near) term neonates (gestational age \geq 35 weeks) with HDFN (due to Rhesus-D, -C, -c, -E, Kell or other red blood cell alloimmunization) treated with IUT and admitted to the Leiden University Medical Center (LUMC) between May 2017 and October 2020 are eligible for the study. The LUMC is the single national referral center in the Netherlands for pregnancies complicated by maternal red blood cell alloimmunization. Approximately 15 eligible patients are treated in the LUMC a year.

Inclusion criteria:

- Gestational age \geq 35 weeks.
- Treatment with IUT due to Rhesus-D, -C, -c, -E, Kell or other red blood cell alloimmunization.
- Birth at the LUMC.

Exclusion criteria:

- Gestational age $<$ 35 weeks.
- Treatment with IUT due to other causes than red blood cell alloimmunization.
- Early onset proven neonatal sepsis.
-

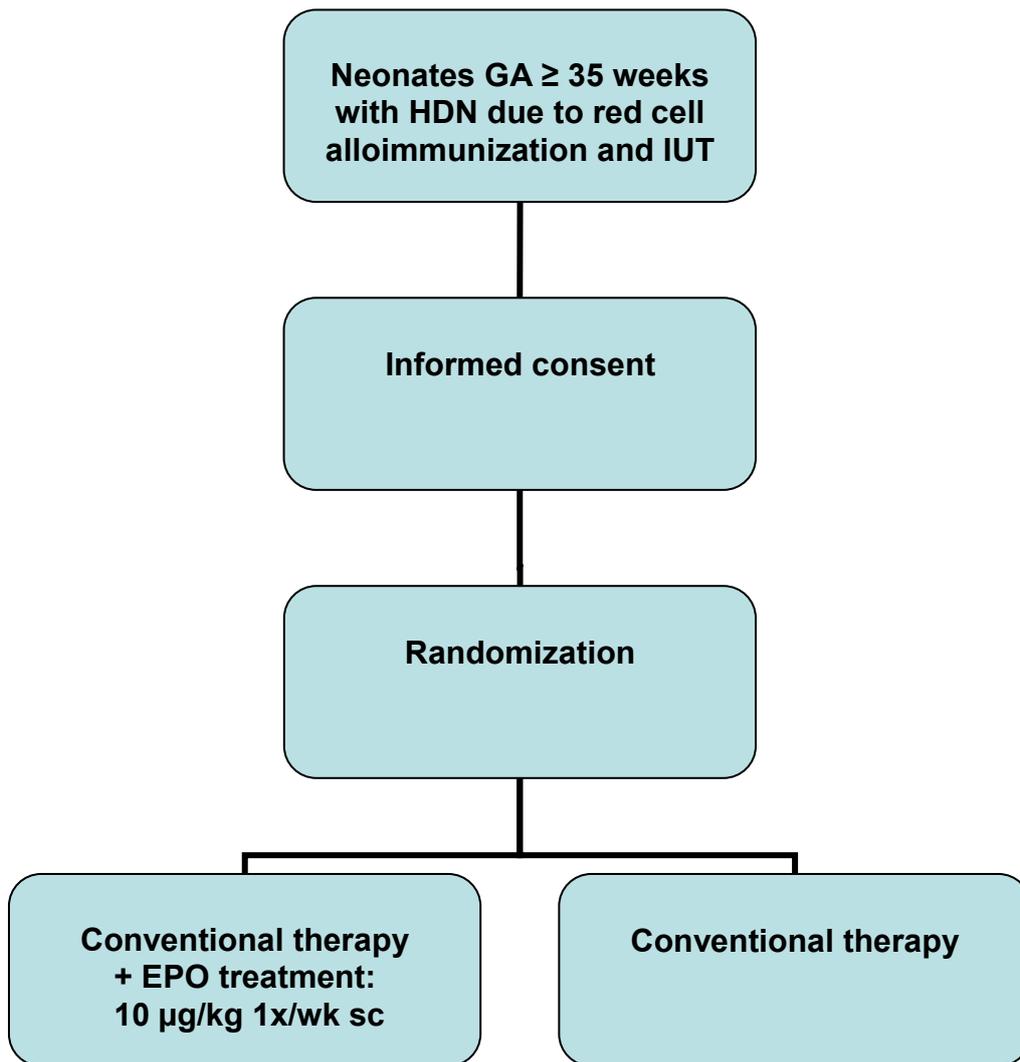
5.2 Study design

Randomized controlled trial. Included neonates will be randomized at birth to treatment with EPO (intervention group) or “standard of care”, 1:1 allocation. In the treatment group, EPO (darbepoetin alfa) is administered subcutaneously at a dosage of 10 μ g/kg once a week, starting at day 7, for a period of 8 weeks. Treatment is administered during weekly home visits after discharge from the LUMC. Concomitant therapy with folate (0.25 mg/day) is given

in both groups (standard practice). Concomitant iron therapy is given if ferritin level drops below 75 microg/l.¹⁹ Weekly routine measurements of complete blood counts (including hemoglobin level, hematocrit and reticulocyte count) will be performed in both groups (standard practice). EPO is discontinued if hemoglobin level is ≥ 13 g/dL after at least 4 weeks of treatment with EPO. Blood pressure will be measured at the start of treatment, after four weeks and after eight weeks. Monthly measurements of liver enzymes (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyl transferase (γ GT) and lactate dehydrogenase (LDH)) will also be performed in all groups (standard practice). In addition, the neonatal EPO-level will be determined at the start of the treatment with EPO. The number of top-up transfusions received during the first 3 months of life and hemoglobin levels prior to the top-up transfusion are recorded. After initial discharge from the LUMC, top-up transfusions are performed when hemoglobin levels fall below 7.2 g/dL or when hemoglobin is between 7.2 and 8.8 g/dL if clinical symptoms of anemia (lethargy, feeding difficulties or failure to thrive) are present.

At two years of age a physical and neurological examination and an assessment of cognitive and neurological development using the Dutch version of the Bayley Scales of Infant Development, third edition (BSID-III) will be performed (standard practice). BSID-III scores provide mental developmental indexes (MDI) and psychomotor development indexes (PDI).

5.3 Flow chart



5.4 Sample size calculation

Based on the (scarce) results in the literature, we expect a 50% reduction in the median number of top-up transfusions per patient with EPO treatment, from a median of 2 to 1. For sample size calculation we hypothesized a shift in the distribution of number of transfusions per infant as depicted in Figure 1. The distribution in the 'care as usual' group are based on data from 2000-2014. Based on these expected frequencies, group sample sizes of 21 achieve 81% power to detect a difference of 1,1 between the null hypothesis that both group means are 1,9 and the alternative hypothesis that the mean of the EPO group is 0,8 with estimated group standard deviations of 1,5 and 0,9 and with a significance level (alpha) of 0,05 using a two-sided Mann-Whitney test. The drop-out percentage is estimated at 5%,

adding $(42/0.95 = 44)$ 1 person to each group's sample size.

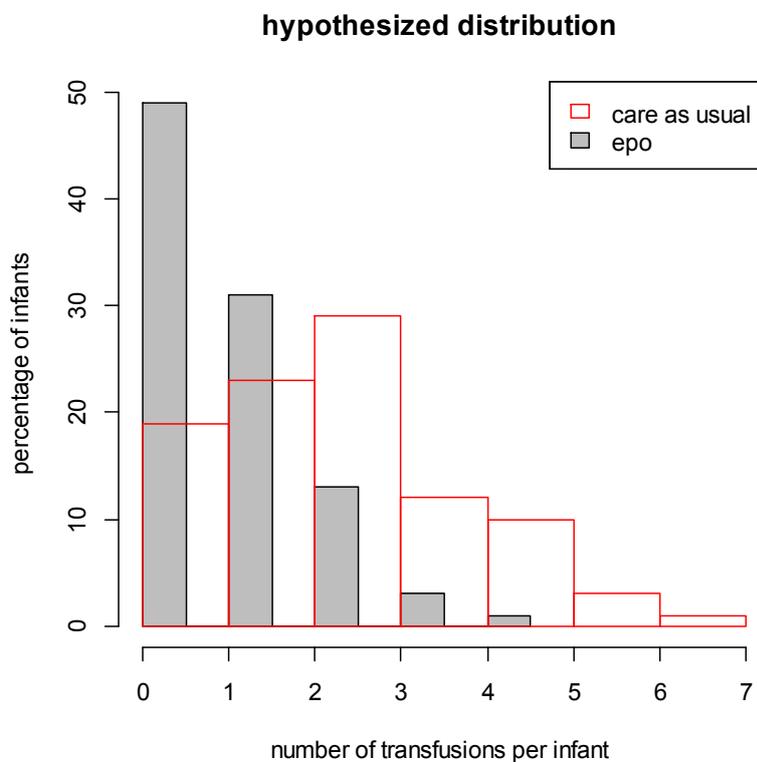


Figure 1. Hypothesized distribution of number of top-up transfusions

5.5 End points

Primary end points:

- Number of top-up transfusions required per infant up to 3 months of life.

Secondary end points:

- The percentage of infants requiring a top-up transfusion up to 3 months of life;
- Time from birth to first top-up transfusion (days);
- Number of days of admission for top-up transfusions;
- The percentage of infants with a systolic blood pressure ≥ 2 SD above age adjusted mean systolic blood pressure during treatment;
- The percentage of infants with ferritin levels >200 $\mu\text{g/L}$ during treatment;

Exploratory end point:

- Long-term neurodevelopmental outcome at 2 years of age using the BSID-III test.

5.6 Randomization, blinding and treatment allocation

Prior to randomization, informed consent will be obtained from parents or caregivers.

Information will be given antenatal if possible to allow sufficient time to consider participation.

Informed consent can also be obtained after birth, but not after the first week of life as not to interfere with the possible darbepoetin treatment schedule. If parents or caregivers agree to participate, treatment will be allocated by the web based randomization service of GRP validated program CASTOR to obtain a unique trial number and assignment of treatment policy. As the primary objectives are based on hemoglobin levels (need for top-up transfusion) that are set out in a transfusion protocol, blinding or the use of placebo is not necessary. Due to the subcutaneous administration route, the use of a placebo is considered unethical.

5.7 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The attending physician can decide to withdraw a subject from the study for urgent medical reasons.

5.8 Replacement of individual subjects after withdrawal

When parents or caregivers want to withdraw their infant for any reason, another infant will be included in the study.

5.9 Cost-effectiveness

By calculating the costs of the intervention (EPO treatment), and taking into account

caregiver time and other services needed and the costs of top-up transfusions, we will assess the cost-effectiveness of the intervention compared to the standard of care. The total costs of EPO treatment using darbepoetin alfa are approximately 32 Euro (total of 8 vials of darbepoetin) per child. The costs of a clinical admission at a day-care-center for one top-up transfusion are approximately 700 Euros. An effective treatment with EPO will reduce the average rate of top-up transfusions from an average of 2 top-up transfusions per child to an average of 1 top-up transfusion per child (absolute reduction of 50%), hereby reducing the costs of treatment by almost 50%, from 1400 Euros (without EPO treatment) to 732 Euros (with EPO).

5.10 Statistical analysis

Both intention to treat and per protocol analysis will be carried out to study the differences in outcome measures between the intervention and control group. Intention to treat analysis is our primary choice of analysis. A minimal treatment with 5 out of 8 darbepoetin injections is defined as acceptable protocol deviation. A p-value < 0.05 will be considered to indicate statistical significance. The number of top-up transfusions per infant and, the number of days of admission for top-up transfusions will be compared between both groups using a Mann-Whitney U test. The time from birth to top-up transfusion will be compared between both groups using survival analysis and a log rank test. The percentage of infants requiring a top-up transfusion up to 2 months of life, the percentage of infants with a high blood pressure and the percentage of infants with high ferritin levels will be compared between both groups using χ^2 tests. If despite randomization the groups differ at baseline in type of alloimmunization, EPO-levels at birth or treatment with exchange transfusion, a multivariable model may be used in a secondary analysis to correct for these factors.

6. REQUIRED CLINICAL EVALUATION

6.1 Maternal and fetal baseline characteristics

The following variables will be recorded: type of red blood cell alloimmunization, course and level of antibody-titers and ADCC (antibody-dependent cell-mediated cytotoxicity test)²⁰ during pregnancy, number of IUTs, fetal hemoglobin values at start of each IUT, presence of severe fetal anemia (< 5 SD), presence of fetal hydrops.

6.2 Neonatal baseline characteristics

The following variables will be recorded: birth weight, gestational age at birth, gender, Apgar score at 5 min, respiratory distress syndrome, patent ductus arteriosus, necrotizing enterocolitis, sepsis and cerebral lesions on cranial ultrasound examination, number of days of phototherapy, number of exchange transfusions, and highest bilirubin level during admission. At birth, the following measurements will be performed in umbilical cord blood as part of standard care: hemoglobin level, hematocrit, reticulocyte count, nucleated red cells (erythroblasts), iron tests (including serum iron, total iron binding capacity, transferrin and ferritin), liver enzymes (ASAT, ALAT, γ GT and LDH). Additionally, EPO-levels will be measured in umbilical cord blood.

6.3 Measurements during follow-up

Complete blood counts including hemoglobin level and reticulocyte count are determined weekly in both groups (standard practice). The number of top-up transfusions received during the first 3 months of life and hemoglobin levels prior to the top-up transfusion are recorded. Iron tests (serum iron, ferritin, transferrin and total iron binding capacity) and liver enzymes (ASAT, ALAT, γ GT and LDH) will be determined monthly (standard practice). Blood pressure will be measured at the start of treatment, after four weeks and after eight weeks of treatment.

7. INVESTIGATIONAL PRODUCT

7.1 Name and description of investigational product

In this study, the investigational medicinal product is darbepoetin alfa (Aranesp). Darbepoetin alfa is a long-acting synthetic substitute for the endogenous hormone erythropoietin that stimulates erythropoiesis. A detailed description of darbepoetin alfa can be found in the summary of product characteristics (SPC) which is added to this protocol as a separate document. The authorized indications of darbepoetin alfa are listed in the SPC on page 5. In children, darbepoetin alfa is used as treatment for symptomatic anemia caused by chronic kidney disease. Relevant information on darbepoetin alfa to treat anemia specifically caused by red cell alloimmunization, is provided in this section. Information is provided on both the erythropoietic and neuroprotective aspects of darbepoetin alfa, as neurodevelopmental outcome will be assessed as a secondary end point.

7.2 Summary of findings from non-clinical studies

Erythropoietic: animal studies in rats and dogs showed a marked increase in hemoglobin levels, hematocrit and reticulocytes after administration of darbepoetin alfa. Adverse effects at high dosages were related to an increased pharmacological effect (decreased tissue perfusion due to increased blood viscosity). Darbepoetin alfa showed no genotoxic or carcinogenic potential, nor had any effect on the proliferation of non-hematological cells *in vitro* or *in vivo* (SPC page 20).

Neuroprotective: both *in vitro* and *in vivo* studies in animal models revealed neuroprotective effects of exogenous EPO administration. These studies showed overall an anti-inflammatory role of EPO and a marked decrease in apoptosis and an increase in neuroregeneration after EPO administration (both recombinant human EPO as well as the long-acting variant darbepoetin alfa) (SPC page 20). Animal studies showed EPO to be able to penetrate the blood brain barrier and showed neuroprotective effects in animal models for neonatal

hypoxic-ischemia and neonatal strokes.^{21,22} A recent study in rats showed neuroprotective effects of EPO (administration of 5000 µg/kg) in induced hemolytic hyperbilirubinemia.²³

7.3 Summary of findings from clinical studies

Erythropoietic: although HDFN is not a registered indication for darbepoetin treatment, off-label use of medication is common in neonatology. The potential effect of EPO in neonates with HDFN was shown in small studies and casuistic reports, showing overall an increased hemoglobin level and decreased transfusion need. No severe adverse effects were reported (dosages 200-400 µg/kg three times a week, recombinant EPO; correlating to a darbepoetin dosage of 5-10 µg/kg once a week).^{9-14,18}

Systematic reviews of both early (27 randomized controlled trials) and late administration (30 randomized controlled trials) of EPO in preterm infants have shown an overall reduction in RBC transfusions. The rates for mortality and morbidities including intraventricular haemorrhage and necrotizing enterocolitis were not significantly changed, EPO administration showed a trend in increased risk for retinopathy of prematurity in this population.^{15,16} A meta-analysis specifically addressing this issue showed no increased risk for any stage of retinopathy of prematurity.^{24,25} EPO has been shown to protect developing retinas in sheep models of endotoxin induced retinal injury and exogenous EPO was found to improve retinal neuronal function in the early stages of retinal ischemia and prevent neovascularization in an animal model of oxygen-induced retinopathy. The previous reported increased risk of retinopathy of prematurity after EPO administration was attributed to the effect of excessive levels of iron, which is toxic to tissues and can cause oxidative damage to lipids, proteins, and DNA.²⁵

Other potential adverse effects include local skin reactions (pain and erythema) due to subcutaneous administration, increased blood pressure with rarely convulsions and hypersensitivity reactions (SPC page 14).

Neuroprotective: the short-term and long-term neuroprotective effects of EPO and specifically of darbepoetin alfa are most widely studied in preterm neonates and term neonates with hypoxic-ischemic encephalopathy. Darbepoetin alfa showed promising results in these groups compared to placebo and appears to be safe (dosage 10 µg/kg once a week).^{21,26,27}

7.4 Summary of known and potential risks and benefits

Darbepoetin alfa is registered as treatment of symptomatic anemia due to chronic kidney disease. As such, it is known that darbepoetin potentially causes local skin reactions, increased blood pressure with rarely convulsions and hypersensitivity reactions. Despite these potential risks, darbepoetin alfa can increase hemoglobin levels and thereby prevent or reduce the amount of top-up transfusions. Potential adverse effects of transfusions would be avoided and the number of days of hospitalization could be reduced. EPO may have an additional beneficial effect on long-term neurodevelopment.

7.5 Description and justification of route of administration and dosage

Darbepoetin alfa is administered subcutaneously, as is common in the registered indications. A dosage of 10 µg/kg once a week for a consecutive period of 8 weeks will be given. The optimal dose of darbepoetin is currently unknown, but the chosen dosage of 10 µg/kg is in agreement with previous studies.^{14,17} A lower dosage is used in anemic patients with chronic kidney disease (SMPC), supporting the probable effectiveness of this dosage to enhance erythropoiesis. When used for its neuroprotective potential, higher dosages are common and are safely used to achieve adequate levels of darbepoetin to pass the blood-brain barrier.²¹

7.6 Preparation and labelling of Investigational Medicinal Product

Preparation and labelling will be done according to relevant GMP guidelines.

Darbepoetin alfa will be provided by the Department of Clinical Pharmacology and Toxicology of the LUMC.

7.7 Drug accountability

Drug accountability will follow current GMP guidelines. The LUMC pharmacy will take full responsibility and supervision of the drug accountability process.

8. SAFETY REPORTING

8.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the parents of the subjects and the accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subjects' health. The investigator will take care that all parents of the subjects are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse and serious adverse events (SAEs)

Adverse events are defined as any undesirable experience occurring to a patient during a clinical trial, whether or not considered related to the investigational drug. All adverse events reported spontaneously by the patient's parents or caregivers or observed by the investigator or his staff will be recorded. Serious adverse events are those which result in:

- Death
- A life-threatening event (i.e. an immediate risk of death)
- Hospitalization or prolongation of hospitalization;
- Severe/permanent disability.

During protocol treatment, all deaths, all SAE's that are life-threatening and any unexpected SAE must be reported to the study coordinator within 48 hours of the initial observation of the event. All details should be documented on the serious adverse event and death report and signed by the responsible investigator or one of the authorized staff members. In circumstances where it is not possible to submit a complete report an initial report may be made giving only the mandatory information.

The investigator will decide whether the SAE is related to the treatment (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be recorded on the serious adverse event form. The assessment of causality is made by the investigator using the following criteria:

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event.
Possible	There is some evidence to suggest a causal relationship (e.g. the event occurred within a reasonable time after administration of the trial medication), but other factors may have contributed to the event (e.g. the patients clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgment of the causal relationship.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events. The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.2.2 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 8.2.1);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in the Investigator's Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorized medicinal product.

The sponsor will report expedited the following SUSARs through the web portal

ToetsingOnline to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal *ToetsingOnline* is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States. This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

8.5 Monitoring

An external monitor will monitor the study and will assess (1) whether the rights and health of the subjects are protected, (2) whether data is correct and verifiable from source documents and (3) whether the study is in agreement with GCP, the approved protocol and legal requirements. A detailed monitor plan is added to this protocol.

8.6 Data safety monitoring board (DSMB)

A DSMB is established to perform ongoing safety surveillance which means that (S)AEs will be monitored. The DSMB will have insight into the total number of (S)AEs and will, as needed, review individual records to be able to analyze potential associations between complications and the study protocol. The members will not have insight in the efficacy data. Accumulating data on (S)AEs will be send to the DSMB for safety review by the researchers. All members have independent positions from the trial study group. The composition and responsibilities of the DSMB are described in detail in the DSMB charter.

The advice(s) of the DSMB will only be sent to the principal investigators of the study. Should they decide not to fully implement the advice of the DSMB, they will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

ETHICAL CONSIDERATIONS

9.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (seventh revision, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

9.2 Recruitment and consent

Parents or caregivers of infants with known red blood cell alloimmunization that were monitored throughout pregnancy for anemia and were treated with IUT(s) for anemia, will be informed (verbally and in writing) about the study by the supervising neonatologist or a member of this research team. Parents can consider their decision at least 24 hours and can ask additional information or questions to an independent doctor before they are asked for their written consent. See appendix A and B (Patient information and Consent form).

9.3 Benefits and risks assessment, group relatedness

HDFN is a condition limited to the neonatal population (group relatedness) Late anemia is a common complication of this condition during the first three months of life, currently treated with top-up transfusions. EPO could potentially be a less invasive alternative to top-up transfusions and be beneficial to our study subjects in terms of reducing the number of needed transfusions.

9.4 Compensation for injury

A liability insurance is obtained in accordance with article 7 of the WMO. This insurance covers:

1. A maximum of €650.000,- for injury or death for each subject who participates in this study;

2. A maximum of €5.000.000,- for injury or death for all subjects who participate in this study;
3. A maximum of €7.500.000,- for the total damage for all subjects of studies by the same organization in each year of insurance coverage.

This insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

9. ADMINISTRATIVE ASPECTS AND MONITORING

10.1 Handling and storage of data and documents

Data management will be implemented according to Good clinical practice (GCP)-guidelines and will comply with the Dutch personal data protection act (Wbp). Data will be handled confidentially and anonymously by coding. The key to the code will be safeguarded by one of the investigators. The coded patient data will be entered in CASTOR (www.castoredc.com), a GCP conform internet based electronic data capture tool secured with login codes. Data will only be transferred encrypted. Data will be stored for 15 years according to the WGBO.

10.2 Monitoring and Quality Assurance

The conduct of the study will be monitored, as is described in detail in the monitoring plan.

10.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

10.4 Annual progress report

The investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events / serious adverse reactions, other problems, and amendments.

10.5 End of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit. In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

10.6 Public disclosure and publication policy

The trial will be registered with European drug regulatory affairs clinical trials (EudraCT). Additionally, the trial will be registered with www.clinicaltrials.gov. Results of the study will be submitted for publication to a peer-reviewed international medical journals.

10. STRUCTURED RISK ANALYSIS

11.1 Potential issues of concern

a. Level of knowledge about mechanism of action

The mechanism of action of EPO in its current indication is well known and detailed in the SPC, page 16. The use of EPO in a neonatal population with HDFN is further described in chapter 7, but overall seems safe with promising clinical results. In this population, affected fetuses are treated with IUTs. Blood transfusions, including IUTs, are known to enhance bone marrow suppression and suppress erythropoiesis.^{4,6} EPO could in turn stimulate erythropoiesis and reduce or treat anemia after birth.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

The effect of the exposure of human beings to darbepoetin alfa is well known as it is a registered product in the treatment of anemia in chronic kidney disease. In patients with HDFN, off-label and/or experimental use has been described with no serious adverse events.^{9-14,18}

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

The working mechanism of EPO and specifically of darbepoetin have been extensively studied in non-clinical settings.^{21,22}

d. Analysis of potential effect

A lower dosage is used in anemic patients with chronic kidney disease (SMPC), supporting the probable effectiveness of this dosage to enhance erythropoiesis. A similar dosage has been administered before^{14,17} and much higher dosages have been safely used when the primary objective was neuroprotection, as EPO only partly crosses the blood-brain barrier.²¹

e. Pharmacokinetic considerations

The half-life of darbepoetin alfa is longer than of recombinant human erythropoietin, justifying a less frequent administering for an equal biologic response. In adults with chronic kidney disease, the half-life is about 73 hours with subcutaneous injections. Pharmacokinetic research in pediatric patients (age 2 to 16 year) with chronic kidney disease, showed similar values (SPC page 19,20).

f. Study population

The study population is by its nature a vulnerable group, consisting of infants with HDFN. Standard procedure in our hospital is to admit neonates with HDFN to the high care unit, where they are clinically and biochemically (bilirubin, hemoglobin) closely monitored and phototherapy treatment is started. Infants randomized to darbepoetin treatment will start treatment after the first week of life for a consecutive period of 8 weeks. All infants with HDFN are followed up to 3 months of age in an out-clinic setting.

g. Interaction with other products

No known interaction is known of darbepoetin alfa with other products (SPC page 12).

h. Predictability of effect

To evaluate the effect of darbepoetin administration, hemoglobin levels will be carefully followed. National transfusion guidelines are followed to determine the need for top-up transfusion bases on hemoglobin cut-offs.

11.2 Synthesis

Good neonatal research is essential if to improve the care and outcome of newborns. The study has been designed to allow parents time to consider participation by approaching them timely for consent. The parents are under no obligation to join the trial and participation is

completely voluntary, as specifically stated in the parent information leaflet.

The results of this study are important to further optimize treatment of HDFN and reduce the transfusion need of this population. Darbepoetin alfa is an approved drug to treat or prevent anemia and did not give rise to any major safety concerns thus far. The study population is a vulnerable group as the patients are newborns, but this research cannot be conducted in another population. Apart from 8 subcutaneous injections with the study medication for half of the study population and an additional visit to assess development of the patients at the age of 2 years for all participants, there is no extra burden for participating infants.

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